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(54) Title: BRANCHED BUILDING UNITS FOR SYNTHESIZING CYCLIC PEPTIDES

(57) Abstract

The present invention relates to building units suitable for the synthesis of backbone cyclized peptides. The building units of this invention have structures of the formula (I): $X-AA_m-U-AA_p-Y$ wherein AA_m and AA_p are residues of natural or synthetic amino acids and U is $C_\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C_\alpha H_2 N)[CO(CH_2)_q W]$, in which C_α is a carbon atom and is the α carbon of AA_m if m > 0. W is NH–X or COY, and X is H, Boc, Z, Fmoc, or Alloc while Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group. The letter m refers to an integer from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6. The present invention is also directed to methods of synthesizing building units of formula (I) via different routes.

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BRANCHED BUILDING UNITS FOR SYNTHESIZING CYCLIC PEPTIDES

1. TECHNICAL FIELD

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The present invention relates to a method for preparing branched building units for use in the synthesis of backbone cyclized peptides, and to the building units themselves.

2. BACKGROUND OF THE INVENTION

Biologically active peptides are conformationally restricted through cyclization to
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achieve metabolic stability, to increase potency, to confer or improve receptor selectivity, and to control bioavailability. The use of medium and long-range cyclization to convert natural bioactive peptides into potential peptidomimetic drugs has been prompted by the ability to control these important pharmacological characteristics. Furthermore, the structural constraints on a peptide brought about by cyclization also enhance conformational
20
homogeneity and facilitate conformational analysis. See, e.g., Kessler, H. Angew. Chem. Int. Ed. Eng. 21:512 (1982). Thus, cyclization may give insight into the biologically active conformations of linear peptides provided that their biological activities and selectivities are maintained.

Common modes of long-range (or global) peptide cyclization include side-chain to
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side-chain, end-group to end-group, and side-chain to end-group, all of which require the alteration or removal of residue side chains. See, e.g., Manesis, N.J., and Goodman, M. Org. Chem. 52:5331 (1987). Backbone cyclization, wherein a connection is made between the N^α and/or C^α atoms of a peptidic backbone, does not require such disruption of the peptide's natural structure because only the hydrogens of the peptide bond are affected. The
30
hydrogens are replaced by ω-functionalized chains that can be interconnected, connected to residue side-chains, or connected to the ends of a peptide to form the desired cyclic peptide. Backbone cyclization can thus stabilize a peptide's bioactive conformation and protect against its enzymatic degradation without altering its side chains.

Although different methods of backbone cyclization exist, a preferred method uses
35
dipeptide building blocks. Gilon and coworkers have disclosed backbone to side-chain and backbone to C-terminus peptide cyclization using lactam and disulfide bridges formed from such building blocks. This was done with the use of N-aminoalkyl amino acids obtained

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either by the alkylation of amino, carboxy or thiol alkyl amines with triflates of α-hydroxy

5   acids, or by the nucleophilic substitution of alkylene diamines. U.S. Patent No. 5,723,575

and Gilon et al., J. Org. Chem. 57: 5687-5692 (1992) (collectively „Gilon").

In the first method of synthesizing N-aminoalkyl amino acids, a diamine is reacted

with an α bromo acid to provide an ω amine which is then selectively protected. Variation

of the protecting group provides a building unit suitable for Boc chemistry peptide

10 synthesis. In the second method of synthesizing these building units, a selectively protected

diamine is reacted with chloracetic acid to provide a protected glycine derivative suitable for

Fmoc peptide synthesis.

In order to take advantage of the facile nucleophilic displacement of carboxylic acid

substituents, both synthetic methods described by Gilon require the reaction of a molecule

15 of formula Hal-CH(R)-CO-OR' (wherein Hal represents a halogen leaving group) and an

amine. The amine bears an alkylidene chain that is terminated by another amine, as shown

in Scheme (I):

20
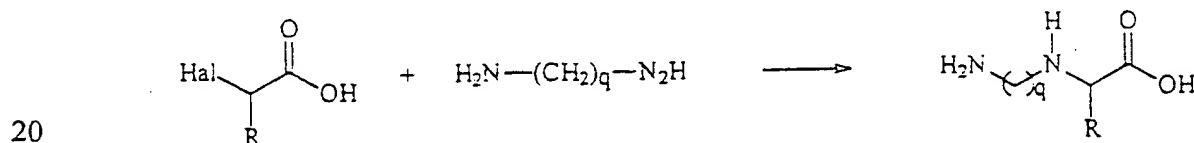
Scheme (I)

The terminating nitrogen atom of the resulting building unit will be contained by the moiety

used to form the bridging chain of a cyclized peptide.

In a molecule where R is other than hydrogen, there is a high tendency to eliminate

25 H-Hal under basic conditions. And because the secondary amine formed by the addition

reaction is a better nucleophile than the primary amine of the diamine reactant, double

alkylation products may form. This side reaction reduces the yield of the method shown in

Scheme (I) to such an extent that it cannot be used for the practical production of building

units based on amino acids other than glycine. Gilon, however, does not suggest backbone

30 cyclization building units having end-group moieties that are not amine, and so only

provides compounds useful in backbone to side-chain and backbone to C terminus peptide

cyclization.

Other workers have described alternative backbone cyclization building blocks. For

example, lactam bridges have been formed with the protected building block

35 N(CH₂COOBu')Phe, although the synthesis of bradykinin analogues using the Boc protected

building block was reportedly hindered by low yields and undesired double couplings.

Reissman et al. Biomedical Peptides, Proteins & Nucleic Acids 1:51-6 (1994). Increased

efficiency was obtained, however, when the protecing group N,O-bis(trimethylsilyl)-

acetamide. The cyclic peptides made with the building block, which contain N-alkylamide

5   bonds, were reported to be unstable under acidic conditions typical of solid phase peptide

synthesis.

The synthesis of backbone cyclized peptides using glycine-based building blocks has

also been reported by Zuckermann et al. J. Am. Chem. Soc. 114: 10646-10647 (1994). This

synthetic approach, which is limited to the solid phase preparation of N-substituted glycine

10   oligomers consists of two steps: first, a resin bound secondary amine is alkylated; and

second, a side-chain is introduced into the polypeptide by nucleophilic displacement of a

halogen with an excess of a primary amine.

A more general method for the synthesis of backbone cyclized peptides is disclosed

by Kaljuste et al. Int. J. Peptide Protein Res. 43: 505-511 (1994). By utilizing amino acid

15   aldehydes, this method allows the formation of branched building units on a solid support,

but requires that both the backbone and the branching chain of the resulting cyclic peptide

contain reduced peptide bonds. The method is further limited by synthetic and storage

problems associated with amino acid aldehydes, the relatively slow rates of alkylation of the

reduced peptide bonds formed during the reaction, and the residue formed from sequence-

20   specific side reactions that can occur during the reductive alkylation of reduced peptide

bonds. Additional purification and racemization problems are also reported.

As made clear above, methods of peptide backbone cyclization have been

constrained by the limited variety of building blocks. For example, the use of similar

building block protecting groups can form hydrophobic clusters along the side chains of a

25   growing peptide which can reduce coupling rates, reaction efficiencies and yields.

Furthermore, some protecting groups can cause the racemization of terminal amino acids

during the synthesis of a cyclic peptide. A third drawback of many building blocks is that

cyclic structures formed from them degrade under the acidic and/or basic conditions typical

of solid phase synthetic methods.

30   There thus exists a need for a larger variety of building units suitable for the

synthesis of cyclic peptides, and a facile and efficient method of synthesizing such building

units. There further exists a need for building units that are stable under solid phase

synthetic conditions.

35   3. SUMMARY OF THE INVENTION

Accordingly, the present invention relates to building units suitable for the synthesis

of backbone cyclized peptides. The building units of this invention have structures of
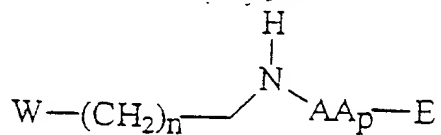
Formula (I):

5   (I)   X-AA$_m$-U-AA$_p$-Y
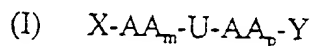
wherein AA$_m$ and AA$_p$ are residues of natural or synthetic amino acids. U refers to $C^\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C^\alpha H_2 N)[CO(CH_2)_q W]$, in which C^α is a carbon atom and is the α carbon of AA$_m$ if m > 0. W is NH-X or COY, and X is H, Boc, Z, Fmoc, or Alloc while Y

10   is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group. The letter m refers to an integer from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6. The present invention also is directed to methods of making building units of Formula (I).

The invention more specifically is directed to a method of synthesizing building

15   units of Formula (II):

(II)   X-AA$_m$C$^\alpha$ON[(CH$_2$)$_{n+1}$-W]AA$_p$-Y

wherein n is a number from 0 to 6. According to this method, a first reaction mixture

20   comprising a compound of Formula (d) is prepared:

$$W\!-\!(CH_2)_n\!-\!\overset{\displaystyle H}{\underset{}{N}}\!-\!AA_p\!-\!E$$

Formula (d)

25   wherein E is a carboxyl protecting group. This reaction mixture is then reacted with a compound of Formula (e):

$$X\!-\!AA_m\!-\!\overset{O}{\underset{}{C}}\!-\!Hal$$

Formula (e)

30   wherein Hal is a halogen, under reaction conditions sufficient to form a second reaction mixture comprising the building unit of Formula (II). The building unit of Formula (II) may then be isolated from the second reaction mixture.

The present invention also encompasses a method of synthesizing a building unit of Formula (III):

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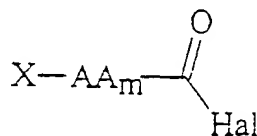(III)   X-AA$_m$$\Psi$(C$^\alpha$H$_2$N)[CO(CH$_2$)$_q$-W]AA$_p$-Y

- 4 -

wherein q is a number from 1 to 6. According to this method, a first reaction mixture comprising a compound of Formula (m) is prepared:
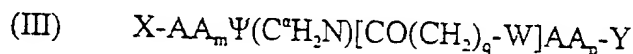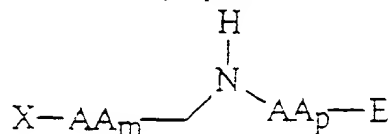
$$\underset{\text{Formula (m)}}{X-AA_m \diagup \overset{\overset{\displaystyle H}{|}}{\underset{}{N}} \diagdown AA_p - E}$$

wherein E is a carboxyl protecting group. This reaction mixture is then reacted with a compound of Formula (n):

$$\underset{\text{Formula (n)}}{W-(CH_2)_q-\overset{\displaystyle O}{\underset{OH}{\diagup\!\!\!\diagdown}}}$$

under reaction conditions sufficient to form a second reaction mixture comprising the building unit of Formula (III). The building unit of Formula (III) may then be isolated from the second reaction mixture.

4. DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

All abbreviations used are in accordance with the IUPAC-IUB recommendations on Biochemical Nomenclature (J. Biol. Chem. 260:14-42 (1983)) and later supplements.

As used herein the term "amino acid" is intended to include any natural or synthetic amino acid. Abbreviations of typical amino acids used in the present invention are provided in Table (I):

Table (I): Amino Acids

Abbreviated Designation	Name
Abu	α-Amino butyric acid
Ala	L-Alanine
Arg	L-Arginine
Asn	L-Asparagine
Asp	L-Aspartic acid
ß Asp (Ind)	ß-Indolinyl aspartic acid
Cys	L-Cysteine

Glu	L-Glutamic acid
Gln	L-Glutamine
Gly	Glycine
His	L-Histidine
Hyp	trans-4-L-Hydroxy Proline
Ile	L-Isoleucine
Leu	L-Leucine
Lys	L-Lysine
Met	L-Methionine
Nal	ß-Naphthyl alanine
Orn	Ornithine
Phe	L-Phenylalanine
Pro	L-Proline
Ser	L-Serine
Thr	L-Threonine
Trp	L-Tryptophane
Tyr	L-Tyrosine
Val	L-Valine

Abbreviations of typical protecting groups, coupling agents, reagents and solvents suitable for use in the present invention are provided in Tables II-IV below. One skilled in the art will understand that the use assigned to each group of compounds is only a common use, and is not intended to limit the ways in which any particular compound may be employed. For example, a compound listed under "reagents and solvents" may be used as a protecting group. Furthermore, it is to be understood that other possible protecting groups, coupling agents, reagents and solvents suitable for use in the present invention are intended to be within its scope.

Abbreviations of protecting groups suitable for use in the present invention are provided in Table (II):

Table (II): Protecting Groups

Abbreviated Designation	Name
Ada	Adamantane acetyl
Alloc	Allyloxycarbonyl
Allyl	Allyl ester
Boc	tert-butyloxylcarbonyl
Bu^t	tert-butyl
Bzl	Benzyl
Fmoc	Fluorenylmethyloxycarbonyl
OBzl	Benzyl ester
OEt	Ethyl ester
OMe	Methyl ester
pixyl	9-phenylxanthen-9-yl
Tos (Tosyl)	p-Toluenesulfonyl
Trt	Triphenylmethyl
Z	Benzyloxycarbonyl

As used herein, the term „coupling agent" refers to any compound or mixture of compounds that facilitates the coupling of two or more compounds. Abbreviations of coupling agents used herein are provided in Table (III):

Table (III): Coupling Agents

Abbreviated Designation	Name
BOP	Benzotriazol-1-yloxytris (dimethyl-amino) phosphonium hexafluorophosphate
DIC	Diisopropylcarbodiimide
HBTU	2-(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
PyBrOP®	Bromotripyrrolidinophosphonium hexafluorophosphate

PyBOP®	Benzotriazol-l-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate
TBTU	O-(1,2-dihydro-2-oxo-1- pyridyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate

Abbreviations of reagents and solvents suitable for use herein are provided in Table (IV):

Table (IV): Reagents and Solvents

Abbreviated Designation	Name
ACN	Acetonitrile
AcOH	Acetic acid
Ac$_2$O	Acetic acid anhydride
AdacOH	Adamantane acetic acid
Alloc-Cl	Allyloxycarbonyl chloride
Boc$_2$O	Di-tert butyl dicarbonate
DCM	Dichloromethane
DMA	Dimethylacetamide
DMF	N,N-dimethylformamide
DIEA	Diisopropylethylamine
Et$_3$N	Triethylamine
EtOAc	Ethyl acetate
Fm	Formyl
FmocOSu	9-fluorenylmethyloxy carbonyl N-hydroxysuccinimide ester
HOBT	1-Hydroxybenzotriazole
HF	Hydrofluoric acid
Me	Methyl
MeOH	Methanol
Mes (Mesyl)	Methanesulfonyl
NMP	1-methyl-2-pyrrolidinone

nin.	Ninhydrin
i-PrOH	Iso-propanol
Pip	Piperidine
PP	4-pyrrolidinopyridine
Pyr	Pyridine
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Triflate (Trf)	Trifluoromethanesulfonyl
Trf₂O	Trifluoromethanesulfonic acid anhydride

As used herein, the term „reaction conditions" refers to conditions under which a chemical reaction may occur, and includes, for example, temperature, time, pressure, catalysts, co-catalysts, solvent, and other variables known to those skilled in the art.

As used herein, the terms „building unit," „building block," „peptide building unit," and „peptide building block" refer to a protected or unprotected compound that contains a moiety which may be incorporated into a peptide or peptide mimetic.

As used here, the terms "stable compound" or "stable structure" refer to a compound that does not undergo substantial decomposition under peptide synthesis conditions.

Because many of the compounds described herein have asymmetric centers, all chiral, diastereomeric, and racemic forms of them are included in the present invention. Many geometric isomers of olefins and the like can also be present in the compounds described herein, and all such stable isomers are contemplated by the present invention.

When any variable, such as X or Y, occurs more than once in any formula herein, its definition in each occurrence is independent of its definition at every other occurrence.

The present invention encompasses building units that enable the backbone cyclization of peptides. Unlike those of the prior art, the building units of this invention typically comprise carboxyl and/or an amino groups sterically hindered only by a branching moiety. In this way, the slow coupling rates typical of prior art building blocks containing, for example, N-alkyl groups may be avoided. An additional advantage of the building units of this invention is that they are easily purified via conventional extraction, precipitation and flash chromatographic means. Furthermore, the building units of the present invention are stable under both acidic and basic conditions, as well as in trimethylsilyl bromide solutions.

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The branched peptide building units of the present invention are preferably formed

5   by one of two methods: the acylation of N-aminoalkyl- or N-carboxyalkyl amino acids, or the acylation of protected pseudopeptides comprising reduced peptide bonds. In general, the individual procedures used to construct the branched peptide building units of this invention rely upon known principles of peptide synthesis, and upon solid phase peptide synthesis in particular. See, e.g., Bodanszky, Principles of Peptide Synthesis (Springer-Verlag: 1984);

10   Bodanszky et al. The Practice of Peptide Synthesis (Springer-Verlag: 1984); Barany and Merrifield The Peptides: Analysis, Synthesis and Biology Vol. 2, Chapter 1 (Academic Press: 1980); Atherton et al. Bioorg. Chem Vol. 8 (1979). For example, synthesis of the compounds of this invention may utilize coupling agents such as those listed above. More novel coupling agents, such as pre-formed urethane-protected N-carboxy anhydrides

15   (UNCA's) and pre-formed acyl fluorides, may also be used. Coupling reactions may occur at room temperature or at elevated temperatures, and in solvents such as, but not limited to, those listed above.

It will be appreciated that in order to achieve good yields of the compounds of this invention, it is generally necessary to protect reactive moieties during synthesis. Suitable

20   protecting groups include those listed above, variations thereof, and additional groups known to those skilled in the art. For example, carboxylic acid end-groups may be protected by the formation of alkyl esters, substituted alkyl esters, thio esters, aryl or substituted aryl esters. As is well known to those skilled in the art, the selection of particular protecting groups used in a synthesis depends on a variety of factors, such as reaction conditions and

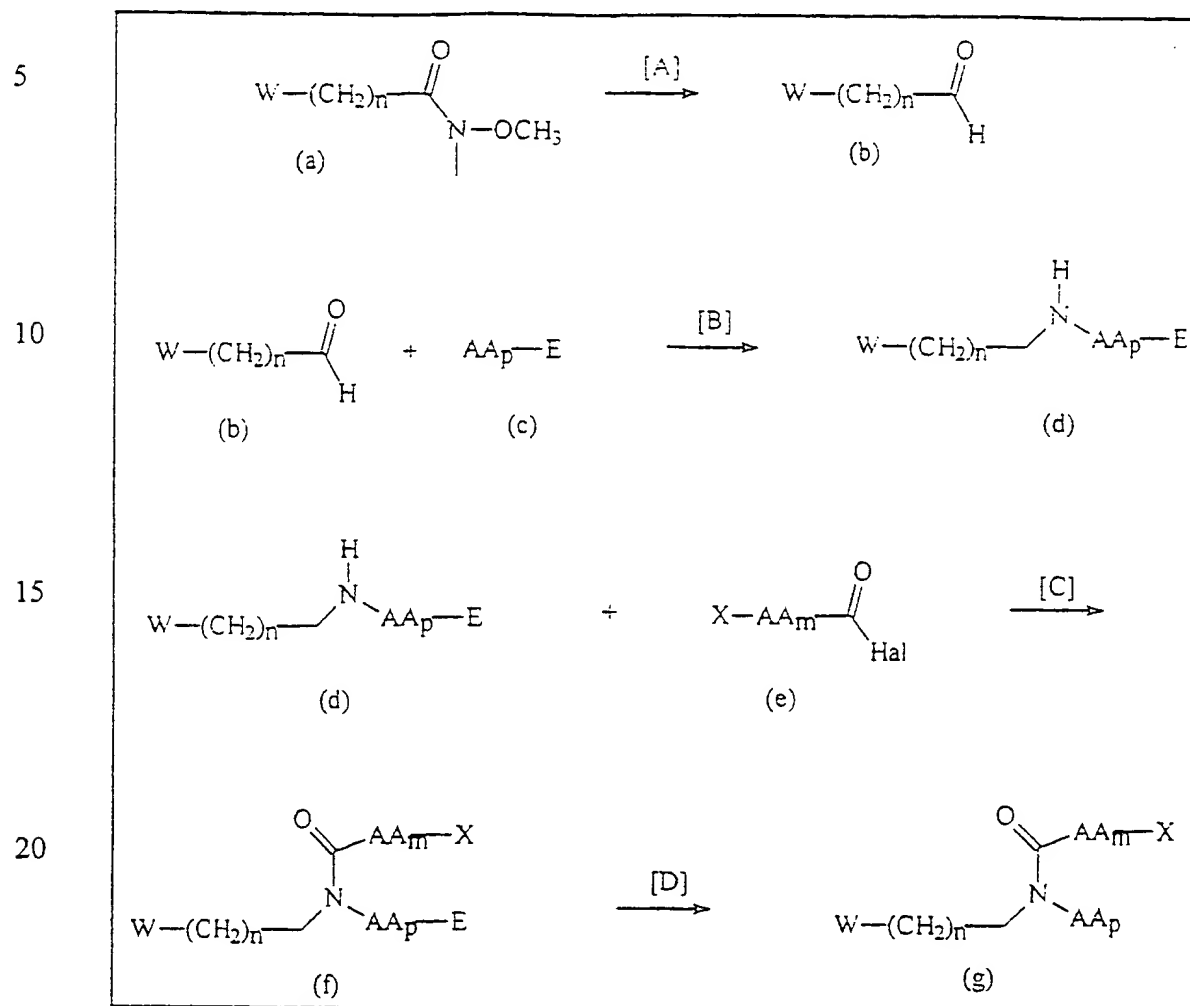25   whether or not other protecting groups are used at the same time.

In the compounds of the present invention, AA_m and AA_p preferably designate residues of naturally occurring or synthetic proteinogenic and non-proteinogenic amino acids. Proteinogenic amino acid residues are those amino acids capable of forming proteins, and include Ala, Arg, Asn, Asp, Cys, Glu, Gln, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser,

30   Thr, Tyr, Val, and derivatives thereof. Although these amino acids may exist in either their L- or D- configurations, the L-configuration is preferred. Additionally, non-proteogenic amino acid residues may be incorporated into branched building units. Although these amino acid residues cannot form proteins on their own, their incorporation into a protein may, for example, aid in determining that protein's active conformers. Reporter groups

35   such as fluorescent amino acids such as 5-hydroxytryptophan, dansyl-lysine, and their derivatives, photolabile amino acid residues, and isotopically labeled amino acids have been used for such purposes. Although these and other non-proteinogenic amino acids may be used in the present invention, AA_m and AA_p are preferably proteinogenic amino acids. AA_m

and AA_p can consist of the same or different types of amino acids. The letter m designates an integer of 0 to 10, preferably 0 to 4, and most preferably 0 or 1, while the letter p designates an integer of 1 to 10, preferably 1 to 4, and most preferably 1 or 2.

The branched building units of this invention possess protected or unprotected amide or carboxylic acid groups. These may be represented by $X\text{-}AA_m$, $AA_p\text{-}Y$, NH-X or COY. X represents an amine or amide hydrogen when a moiety such as the N terminus of the terminal AA_m group or the artificial N terminus formed by U when m = 0 is unprotected. Similarly, when a carboxylic acid moiety is unprotected, Y represents the hydroxyl group of the acid. When the amine or amide to which X is attached is protected, X represents a suitable protecting group such as, but not limited to, Boc, Z, Fmoc or Alloc. When a carboxylic acid moiety is protected, Y represents a suitable protecting group such as, but not limited to, alkoxy, substituted alkoxy, allyloxy, substituted allyloxy, aryloxy, and trimethylsilyl ethers; specific examples include OMe, OBzl, OFm, OAll, and OBu^t. These protecting groups may be covalently attached to an insoluble polymeric support.

The moiety represented by U in Formula (I) refers to either $C^\alpha ON[(CH_2)_{n-1}W]$ or $\Psi(C^\alpha H_2 N)[CO(CH_2)_q W]$, wherein W is NH-X or COY. C^α represents a carbon atom, and the α carbon of AA_m if m > 0; $\Psi(C^\alpha H_2 N)$ represents a reduced carbon-nitrogen bond, and a reduced peptide bond between adjacent AA_m and AA_p residues if m > 0. The letter n designates an integer from 1 to 6, preferably an integer from 1 to 4 and the letter q also designates an integer from 1 to 6, preferably an integer from 1 to 4.

The building units of Formula (II) are preferably branched dipeptides. Although these dipeptides may be synthesized by any means known to those skilled in the art, they are preferably synthesized by an approach wherein an amino acid ester is reductively alkylated to yield a N-aminoalkyl- or N-carboxyalkyl amino acid, which is subsequently coupled with a halogenated amino acid. A preferred embodiment of such an approach is shown in Scheme (II):

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SUBSTITUTE SHEET (RULE 26)

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W—(CH₂)n—C(=O)—N(—OCH₃)   —[A]→   W—(CH₂)n—C(=O)H

(a)   (b)

10

W—(CH₂)n—C(=O)H   +   AAp—E   —[B]→   W—(CH₂)n—CH₂—N(H)—AAp—E

(b)   (c)   (d)

15

W—(CH₂)n—CH₂—N(H)—AAp—E   +   X—AAm—C(=O)Hal   —[C]→

(d)   (e)

20

O=C—AAm—X, W—(CH₂)n—N(—AAp—E)   —[D]→   O=C—AAm—X, W—(CH₂)n—N(—AAp)

(f)   (g)

Scheme (II)

This approach reflects the inventors' discovery that benzyl and tertiary butyl protecting
groups can form hydrophobic clusters that restrict the variety of solvents and reactants that
may be used to form the building blocks of the present invention.

The reaction conditions represented in Scheme (II) by [A], [B], [C] and [D] may be
altered or changed in ways understood by those skilled in the art, and will vary depending
upon the particular reactants used and whether the reactions are performed in the liquid or
solid phase. Preferably, however, the conditions are as follows: [A] represents reductive
conditions suitable for the formation of a compound of Formula (b), and includes the use of,
for example, (iso-Bu)$_2$AlH, LiAlH(O-Bu^t)$_3$, LiAlH$_4$-EtOH, NaAlH$_4$, and diaminoluminum
hydrides; [B] represents conditions suitable for the reductive alkylation of the compound of
Formula (c), and includes the use of catalysts such as H$_2$/Ni, NaCNBH$_3$, and
BrCH$_2$COOBu^t/Ag$_2$O; [C] represents conditions suitable for the coupling of the compounds
of Formulas (d) and (e), and includes the use of a coupling agent or catalyst such as
collidine, BTSA, and BTSA in combination with DIEA; and [D] represents conditions
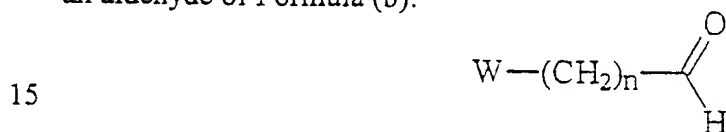
suitable for the deprotection of the compound of Formula (f), and includes the use of an acid

5   such as acetic acid, hydrochloric acid, and sulfuric acid in the presence of a catalyst such as
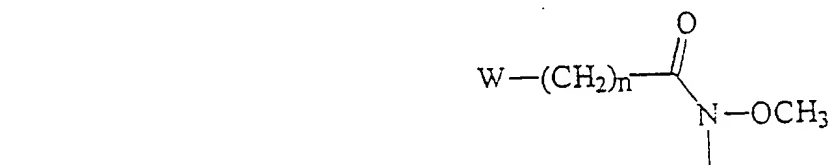
palladium acetate.

        Although the method shown in Scheme (II) is a preferred method of forming

building blocks of Formula (I), it is to be understood that variations of this method are

encompassed within the present invention. For example, the intermediate of Formula (d)

10 may be deprotected prior its coupling with the compound of Formula (e).

        As shown in Scheme (II), an amino acid ester of Formula (c), which is preferably a

trimethyl silyl or tertiary butyl ester of an N-alkyl amino acid, is reductively alkylated with

an aldehyde of Formula (b):

15

$$W-(CH_2)_n-\overset{\displaystyle O}{\overset{\|}{C}}-H$$

Formula (b)

wherein W represents a protected end-group, preferably a protected amide or carboxylic

acid, and the protecting moiety is preferably Alloc or OAll. The aldehyde of Formula (b) is

20 preferably, but not necessarily, obtained via the reduction of an N-alkoxyamide. A preferred

N-alkoxyamide is the N-methoxyamide of Formula (a):

$$W-(CH_2)_n-\overset{\displaystyle O}{\overset{\|}{C}}-\underset{|}{N}-OCH_3$$

25   Formula (a)

Reaction conditions [A] suitable for such a reduction are known to those skilled in the art,

and include the use of reductants such (iso-Bu)$_2$AlH, LiAlH(O-Bu^t)$_3$, LiAlH$_4$-EtOH,

NaAlH$_4$, and diaminoluminum hydrides, although LiAlH$_4$-EtOH is preferred. After much

investigation, the present inventors have learned that the Alloc and OAll protecting groups

30 are not readily reduced under such conditions, nor are they readily reduced during the
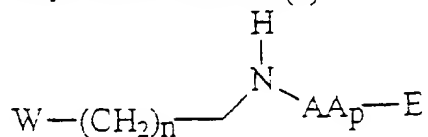
following reductive alkylation reaction.

        Next, according to Scheme (II), the aldehyde of Formula (b) is reacted with a

protected amino acid or peptide ester of Formula (c):

35   AA_p-E

                             Formula (c)

wherein E represents a carboxyl protecting group such as alkoxy, substituted alkoxy,

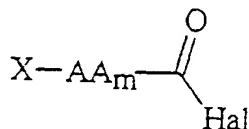allyloxy, substituted allyloxy, aryloxy, or trimethylsilyl ether, any of which may be

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covalently attached to an insoluble polymeric support. Preferred protecting groups include

5   OMe, OBzl, OFm, OAll, and OBu'. This reaction is run under reaction conditions [B] that

enable the formation of a compound of Formula (d):

$$
\begin{array}{c}
\text{H} \\
| \\
\text{N} \\
\end{array}
$$

W—(CH$_2$)$_n$——__/N_AA$_p$—E

10   Formula (d)

The reaction is preferably run in one of the above disclosed solvents, although DMF is most

preferred, and a catalyst is used. Suitable catalysts are known to those skilled in the art and

include, for example H$_2$/Ni, NaCNBH$_3$, and (BrCH$_2$COOBu')/Ag$_2$O. Preferably, the catalyst

is NaCNBH$_3$. After the catalyst is added, the solution is stirred until the reaction is

15   complete; a duration of 24 hours at room temperature is typically sufficient. The protected

N-alkylene amino acid product of Formula (d) is then isolated from the reaction mixture

using conventional extraction techniques.

        The N-alkylene amino acid product of Formula (d) is then coupled with a protected

amino acid halide of Formula (e):

$$
\begin{array}{c}
\text{O} \\
\| \\
\end{array}
$$

20   X—AA$_m$——____

                                    Hal

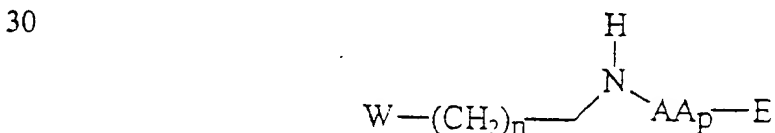   Formula (e)

If Hal is Cl, it is preferred that the reaction conditions represented by [C] in Scheme (II)

include the use of DCM as the solvent, and a coupling agent such as one of those disclosed

25   above. It is preferred, however, that the reaction be catalyzed by BTSA alone or in

combination with DIEA. If this combination is used, it is further preferred that BTSA is

added to a solution of the N-alkylene amino acid Formula (d) at about room temperature

over the course of about 24 hours. The solution is preferably then cooled to about 0 °C, at

which point DIEA is added. At this time, the protected amino acid chloride of Formula (e)

30   is added to the solution while the solution is stirred vigorously. Preferably, the protected

amino acid chloride is added to the solution in five equal portions. Stirring is continued at

about 0 °C for about 1 hour, and continues after the solution temperature is raised to about

room temperature for about another 24 hours or at least until some of the dipeptide building

units having Formula (f) are formed. The branched dipeptide building unit may then

35   deprotected prior to isolation and purification, simply isolated and purified, or isolated and

purified and then deprotected to yield building blocks of the present invention. It should be

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noted that Formula (II) encompass both protected compounds of Formula (f) and
5   deprotected compounds of Formula (g).

As indicated by Scheme (II), the compound of Formula (e) need not be a protected
amino acid chloride. Indeed, the present inventors have learned that the N-terminal amino
acid of the protected dipeptide of Formula (f) can be racemized when the reactant of
Formula (e) is an acid chloride. The present inventors have also found, however, that this
10   problem may be avoided if the compound of Formula (e) is a protected amino acid fluoride
(i.e., Hal = F). Specifically, it has been discovered that dipeptides of Formula (f) may be
obtained in good yield and with a minimum of racemization from the reaction of a Fmoc-
AA-fluoride with an N-alkyl amino acid tertiary butyl ester. Without being limited by
theory, this is believed to result from the enhanced stabilities of carboxylic acid fluorides as
15   compared to their corresponding chlorides. Furthermore, because the N-alkyl amino acid
ester of Formula (c) is more nucleophilic than the free carboxylic group, the fluoride
compounds are activated to an extent sufficient to provide good coupling rates. Because
different carboxylic acid halides react differently, the reaction conditions represented by [C]
in Scheme (II) are preferably different than those described above for Hal = Cl.
20   Specifically, BTSA is preferably replaced with the weaker base collidine, which is less
likely to lead to the formation of undesired side-products.

The coupling reaction yields a protected dipeptide building block of Formula (f)
which may be deprotected by conventional means. Specifically, reaction conditions
represented by [D] in Scheme (II) include those that hydrolyze esters. For example, an
25   organic acid such as acetic acid or a mineral acids such as hydrochloric acid and sulfuric
acid may be used in the presence of a catalyst such as palladium acetate to remove the
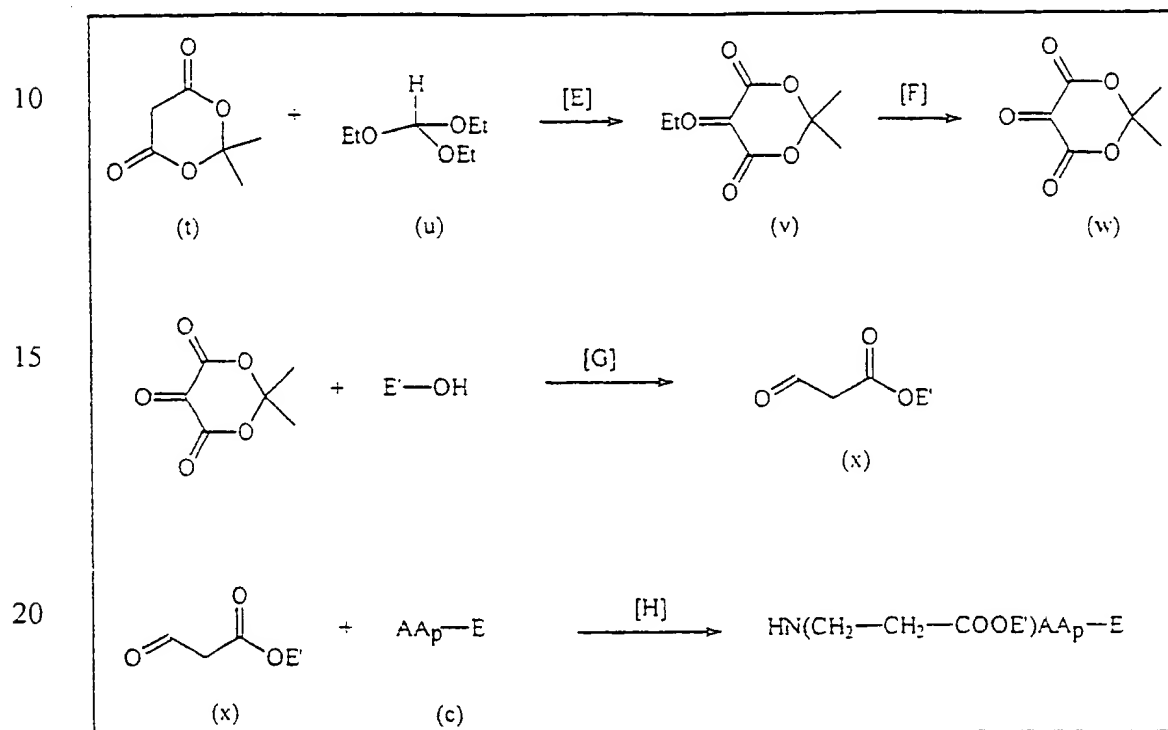protecting group Y.

As described above, the building blocks of Formula (II) are preferably synthesized
from N-aminoalkyl or N-carboxyalkyl amino acids of Formula (d):

30

$$W-(CH_2)_n \overset{\overset{\displaystyle H}{\underset{\displaystyle |}{N}}}{\diagup} AA_p - E$$

Formula (d)

wherein W is HNX or COY. N-carboxymethyl amino acids may thus be used in the
35   synthesis of the building units of this invention, although N-carboxymethyl amino acids
tend to form dioxopiperazines under reaction conditions designated by [C] in Scheme (II).
The present inventors have unexpectedly found, however, that the simple extension of the
alkyl chain from methyl to ethyl avoids this problem. Accordingly, when Formula (d)

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represents a N-carboxyalkyl amino acid, it is preferred that it not be a N-carboxymethyl

5   amino acid. It is more preferred that it be a N-carboxyethyl amino acid, which may be

synthesized by means other than those shown in Scheme (II). A method of preparing N-

aminoalkyl amino acids of Formula (d) that has been found particularly useful in the present
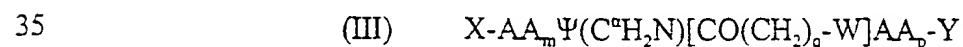

Scheme (III)

invention utilizes Formyl-Meldrum's acid as shown in Scheme (III):

25 wherein E and E' are carboxyl protecting groups. Suitable carboxyl protecting groups

include, but are not limited to, alkoxy, substituted alkoxy, allyloxy, substituted allyloxy,

aryloxy, and trimethylsilyl ethers; specific examples include OMe, OBzl, OFm, OAll, and

OBu^t.

30

In another embodiment, the present invention encompasses peptide building units of

Formula (III):

35   (III)   X-AA$_m\Psi$(C$^\alpha$H$_2$N)[CO(CH$_2$)$_q$-W]AA$_p$-Y

These compounds differ from those of Formula (II) in that they contain a reduced peptide

bond between adjacent AA$_m$ and AA$_p$ residues when m > 0, and an amide bond to the

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branching moiety. The reduced peptide bond is indicated by $\Psi(C^{\alpha}H_2N)$. Building units of

5   Formula (III) allow more combinations of different protecting groups than those of Formula

(II), yet exhibit similar stability in acidic and basic environments.

Although building units of Formula (III) may be synthesized by any means known to

those skilled in the art, it is preferred that they be synthesized according to the method

shown in Scheme (IV). Advantages of this method include high yields and limited
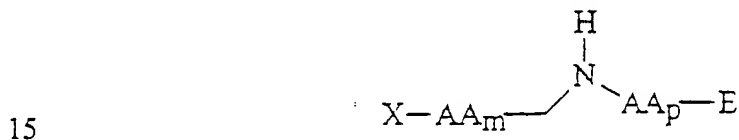
10

Scheme (IV)

formation of undesirable side-products such as dioxopiperazines.

The approach shown in Scheme (IV) is based on an understanding of how aldehyde

derivatives of natural and synthetic amino acids may be used to form dipeptides comprising

reduced peptide bonds. This approach, like that shown for the first embodiment of the
30

invention, is also based upon the inventors' discovery of novel ways by which aldehydes

may be synthesized from protected amino acids, and from Fmoc-protected amino acids in

particular. The synthesis of compounds of Formula (k) is thus preferably the same as that of

Formula (b) shown in Scheme (II).

35   The reaction conditions represented in part by [J], [K] and [L] may be altered or

changed in ways understood by those skilled in the art, and will vary depending upon the

particular reactants used and whether the reactions are performed in the liquid or solid

phase. Preferably, however, the conditions are as follows: [J] represents reductive

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conditions suitable for the formation of a compound of Formula (m), and includes the use

5   of, for example, catalysts such as H_2/Ni, $NaCNBH_3$, and $BrCH_2COOBu^t/Ag_2O$; [K]

represents conditions suitable for the coupling of the compounds of Formulas (m) and (n),

and includes the use of a coupling agent or catalyst such as collidine, BTSA, and BTSA in

combination with DIEA; and [L] represents conditions suitable for the deprotection of the

compound of Formula (o), and includes the use of an acid such as acetic acid, hydrochloric

10   acid, or sulfuric acid in the presence of a catalyst such as palladium acetate.

According to Scheme (IV), an amino acid aldehyde of Formula (k) is coupled with a

protected amino acid of Formula (l) to yield a pseudopeptide of Formula (m):

$$X-AA_m \diagup \underset{N}{\overset{H}{|}} \diagdown AA_p-E$$

15

Formula (m)

wherein E is a carboxyl protecting group. Suitable carboxyl protecting groups include, but

are not limited to, alkoxy, substituted alkoxy, allyloxy, substituted allyloxy, aryloxy, and

trimethylsilyl ethers; specific examples include OMe, OBzl, OFm, OAll, and OBu^t.

20   Preferably, the reaction conditions represented by [J] include the use of a catalyst such as

H_2/Ni, $NaCNBH_3$, or $BrCH_2COOBu^t/Ag_2O$, although other catalysts may of course be used.

It has been found that the reaction proceeds smoothly if the solvent is DMF.

The compound of Formula (m) is then coupled with a carboxylic acid derivative of

25

$$W-(CH_2)_q-\overset{O}{\overset{\diagup\!\!\diagdown}{C}}-OH$$

Formula (n)

Formula (n):

under reaction conditions represented by [K] in Scheme (IV). Preferably, this reaction is

30   done in DMF with the aid of a coupling agent added after the reactant mixture has been

cooled to about 0°C. Suitable coupling agents which may then be added include, for

example, collidine, HBTU, HOBT, BTSA, and BTSA in combination with DIEA. After all

the reactants have been combined, the resulting solution is preferably stirred for about one

hour at 0°C and then overnight at room temperature or at least until branched peptides of

35   Formula (o) are formed. Because the product of this above reaction is crude, it is preferred

that the reaction products are recoupled with another coupling agent, preferably PyBrOP®.

The reaction product may be extracted from the solution using, for example,
5   crystallization, precipitation, or flash chromatographic means. If desired, the reaction
product is then dissolved in acid, preferably acetic acid, along with a hydrolyzation catalyst
such as palladium acetate to form deprotected branched dipeptides of Formula (p).

The building units of Formula (I), which encompasses those of Formulas (II) and
(p), may be used in conventional solid phase peptide synthesis, and thus may be bound to
10   other amino acids of an overall peptide structure. The coupling rates of these reactions may
be monitored using quantitative tests for free amine groups and by the removal of UV
detectable amino protecting groups.

5. EXAMPLES

15   The invention is further defined by reference to the following nonlimiting examples.
The compounds described in these examples were analyzed using a variety of techniques
including thin layer chromatography (TLC), high pressure liquid chromatography (HPLC),
and fast atomic bombardment mass spectroscopy (FAB-MS). Thin layer chromatography
was conducted using silica gel 60, F254, Merck 0.25 mm glass plates using the following
20   solvent systems: S_1 = hexane-ethyl acetate 1/1 v/v; S_2 = chloroform-methanol 7/3 v/v; S_3 =
chloroform-methanol 9/1 v/v; S_4 = chloroform-methanol-acetic acid 90/8/2 v/v/v; S_5 = n-
butanol-acetic acid-water 4/1/1 v/v/v; S_6 = benzene-acetone-acetic acid 27/10/0.5 v/v/v.
HPLC measurements utilized a Knauer Column (220 nm, HD-SIL-18-5s-80A, 250x4 mm)
with a gradient that began at from 20% acetonitrile in 0.1% aqueous TFA and progressed to
25   80% acetonitrile at a rate of 1%/min; a flow rate of 1.0 ml/min was used. These examples
are representative, and should not be construed to limit the scope of the present invention.

Example 1: H-N(CH₂COOBuᵗ)Phe-OMe

H-Phe-OMe·HCl (2.16 g/ 10 mmol) was dissolved in 50 ml freshly distilled DMF.
30   Tertiary butyl bromoacetate (2.44 g/ 12.5 mmol) and silver(I)oxide (5.79 g/ 25 mmol) were
added under stirring at room temperature. Stirring was continued for 24 hours during whcih
the color of the silver oxide changed from black to grey. The reaction process was
monitored by TLC (system 1/ ninhydrine): the starting material H-Phe-OMe·HCl
disappeared as a new, main spot was detected. After reaction completion, the mixture was
35   filtered through a silica gel bed to remove the silver salts. The precipitate was washed with
DMF (3x10 ml) and the filtrates were evaporated to dryness under vacuum. The residue
was dissolved in ethyl acetate (50 ml) and extracted with 5% $KHSO_4$ (3x10 ml), 10%
$NaHCO_3$ (3x10 ml) and saturated aqueous NaCl. After drying with anhydrous Na_2SO_4 the

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solvent was removed under vacuum. The resulting oil (2.12 g/ 72% yield) was pure by TLC

5   and HPLC. No H-Phe-OMe·HCl was detected (HPLC t_R 5.10 min) and no diketopiperazine

formation was observed. After acetylation with Ac_2O/ DIPEA and saponification by LiOH/

THF at 25C°, the spot (TLC) of the product disappears within 90 minutes.

Yield:	2.12 g (72%)
	$C_{16}H_{23}NO_4$ M.W. = 293.3
	$[\alpha]D$ = n.d.
TLC Results:	R_f:(S1) 0.68 (benzene:acetone:acetic acid 27:10:0.5)
	R_f:(S2) 0.82 (chloroform:methanol/ 9:1)
	R_f:(S3) 0.56 (hexane:ethyl acetate/ 1:1)
HPLC Results:	t_R 17.54 min
EI-MS:	m/e[MH⁻] 294.3

Example 2: Fmoc-D-Phe-Ψ[CO-N(CH$_2$COOBu^t)]Phe-OH

A mixture of HN(CH$_2$COOBu^t)Phe-OH (0.279 g, 1.0 mmol) in DCM (10 ml) was

treated with BTSA (0.98 ml, 4 mmol) at room temperature for 24 hours. The clear solution

20   was cooled to 0 °C and DIEA (0.21 ml, 1.25 mmol) was added. The reaction mixture was

stirred vigorously and Fmoc-D-Phe-Cl (0.507 g, 125 mmol) was added in five portions.

Carpino, L. A. et al., J. Org. Chem. 51:3732-3734 (1986). Stirring was continued for 1 hour

at 0 °C and then 24 hours at room temperature. The solvent was removed and the residue

taken up with ethyl acetate (30 ml), washed with 0.1 N KHSO$_4$ (3x10 ml), saturated with

25   aqueous NaCl solution (3x10 ml), dried over Na$_2$SO$_4$; and evaporated to dryness. The

residue was purified by flash chromatography on silica gel 60 (chloroform/methanol 94/6).

Still W. C. et al. J. Org. Chem. 14:2923-2925 (1978). The fractions containing pure

products were combined, evaporated and dried under vacuum.

Yield:	0.305 g (0.47 mmol) 47.0%	
	$C_{39}H_{40}N_2O_4$	M.W.= 648.73 g/mol
	$[\alpha]_D$:	-3.5°(c=1.0 MeOH)
TLC Results:	R_f:(S3)	0.77
	R_f:(S4)	0.86
HPLC Results:	t_R 54.2 min	
FAB-MS:	631 ((M-H$_2$O)⁻), 650 ((M+1)⁻)	

Example 3: Fmoc-D-Phe-Ψ[CO-N(CH₂CH₂NHBoc)]Phe-OH

5   A suspension of HN(CH₂CH₂NHBoc)Phe-OH (0.308 g, 1.0 mmol) in DCM (10 ml) was treated with BTSA (0.98 ml, 4 mmol) at room temperature for 24 hours. The clear solution was cooled to 0 °C and DIEA (0.34 ml, 2.0 mmol) was added. The reaction mixture was stirred vigorously and Fmoc-D-Phe-Cl (0.507g, 1.25mmol) was added in five portions within 20 minutes. Carpino et al. J. Org. Chem. 51:3732-3734 (1986). Stirring

10   was continued for 1 hour at 0 °C and then 24 hours at room temperature. The solvent was removed and the residue taken up with ethyl acetate (30 ml), washed with 0.1 N KHSO₄ (3x10 ml), saturated aqueous NaCl solution (3x10 ml), dried over Na₂SO₄; and evaporated to dryness. The residue was purified by flash chromatography on silica gel 60 (chloroform/methanol 92/8). Still et al. J. Org. Chem. 14:2923-2925 (1978). The fractions

15   containing pure product were combined, evaporated and dried under vacuum to yield a pale yellow oil.

Yield:	0.293 g (0.43 mmol) 43.3%	
	$C_{40}H_{43}N_3O_7$	M.W.= 677.77 g/mol
	$[\alpha]_D$:	-21.4°(c=1.0 MeOH)
20 TLC Results:	R_f:(S3):	0.45
	R_f:(S4):	0.52
HPLC Results:	t_R:	49.4 min
FAB-MS:	578 ((M+1-Boc)⁻), 678 (M⁺), 716 ((M-1+K)⁺)	

25   Example 4: Fmoc-ValΨ[CO-N(CH₂COOBuᵗ)]Phe-OH

A mixture of HN(CH₂COOBuᵗ)Phe-OH (2.0 g, 7.16 mmol) in DCM (100 ml) was treated with BTSA (7.00 ml, 28.64 mmol) at room temperature for 24 hours. The clear solution was cooled to 0 °C and DIEA (1.425 ml, 8.59 mmol) was added. The reaction mixture was stirred and Fmoc-Val-Cl (2.55 g, 7.16 mmol) was added in five portions.

30   Stirring was continued for 1 hour at 0°C and then for 24 hours at room temperature. The solvent was removed and the residue taken up with ethyl acetate (100 ml), washed with 0.1M KHSO₄ (3x30 ml), saturated aqueous NaCl solution (3x30 ml), dried over Na₂SO₄; and evaporated to dryness. The residue was purified by flash chromatography on silica gel 60(chloroform/methanol 94/6). The fractions containing pure product were combined,

35   evaporated and dried under vacuum.

Yield:	3.4 g (79%) (without flash chromatography)
	1.13 g (27%) (after flash chromatography)
	$C_{35}H_{40}N_2O_7$ M.W. 600.69

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TLC Results: R_f:(S$_3$) 0.83

5   R_f:(S$_4$)   0.71

HPLC Results: t_R: 51.2 min

FAB-MS: 601 (M+1)$^+$

Example 5: Fmoc-ValΨ[CO-N(CH$_2$-CH$_2$-CH$_2$-NHBoc)]Phe-OH

10   A suspension of HN(CH$_2$-CH$_2$-CH$_2$-NHBoc)]Phe-OH (322 mg, 1.0 mmol) in DCM

(20 ml) was treated with N,O-bis(trimethylsilyl)acetamide (980 µl, 4.0 mmol) at room

temperature for 20 hours. The clear solution was cooled to 0 °C and DIEA (340 µl, 2.0

mmol) was added. The reaction mixture was stirred and Fmoc-Val-Cl (446 mg, 1.25 mmol)

added over the course of 20 minutes. Stirring was continued for 1 hour at 0 °C and then for

15 24 hours at room temperature. The solvent was removed and the residue taken up in ethyl

acetate (100 ml), washed with 0.1M KHSO$_4$ (3x30 ml), saturated aqueous NaCl solution

(3x30 ml), dried over Na$_2$SO$_4$; and evaporated to dryness. The crude product were purified

by flash chromatography on silica gel 60 (chloroform/methanol 91/9). The fractions

containing pure product were combined, evaporated and dried under vacuum to yield a pale

20 yellow oil.

Yield: 550 mg (85%)   (crude product)

                        C$_{37}$H$_{45}$N$_3$O$_7$ M.W. 643.79

TLC Results: R_f:(S$_2$) 0.63

HPLC Results: t_R: 48.50 min

25 FAB-MS: 644 (M$^+$)

Example 6: Fmoc-ValΨ[CO-N(CH$_2$-CH$_2$-NHBoc)]Phe-OH

A suspension of HN(CH$_2$-CH$_2$-NHBoc)]Phe-OH (925 mg, 3.0 mmol) in DCM (20

ml) was treated with N,O-bis(trimethylsilyl)acetamide (2.94 ml, 12 mmol) at room

30 temperature for 20 h. The clear solution was cooled to 0 °C and DIEA (1.02 ml, 6 mmol)

was added. The reaction mixture was stirred and Fmoc-Val-Cl (1.34 g, 3.75 mmol) added in

some portions within 20 min. Stirring was continued for 1 hour at 0 °C and 24 hours at room

temperature. The solvent was removed and the residue taken up in ethyl acetate (100 ml),

washed with 0.1M KHSO$_4$ (3x30 ml), saturated aqueous NaCl solution (3x30 ml), dried

35 over Na$_2$SO$_4$; and evaporated to dryness. The crude product were purified by flash

chromatography on silica gel 60 (chloroform/methanol 91/9). The fractions containing pure

product were combined, evaporated and dried under vacuum to yield a pale yellow oil.

Yield: 1.5 g (80%)   (crude product)

$C_{36}H_{43}N_3O_7$ M.W. 629.76

5 TLC Results: R_f:(S₂) 0.72

   HPLC Results: t_R: 48.36 min

   FAB-MS: 630 (M⁺)

Example 7: Boc-βAla Ψ(CH₂-NH)Phe-OH

10   Boc-βAla Ψ(CH₂-NH)Phe-Obzl (5.0 g, 12.12 mmol) was hydrogenated in aqueous acetic acid (90%, 50 ml) with Pd-acetate (250-500 mg) for 12 hours. The reaction was monitored by TLC in hexane-ethylacetate 1/1. The catalyst was filtrated through a layer of silica gel (Merck, 0.04-0.063 mm), and the palladium was washed black with acetic acid (3x30 ml). The filtrates were separately evaporated under vacuum to dryness. To remove

15 impurities, the substances was washed intensively with ether and water to catch the phenylalanine, and then evaporated to dryness with ether.

   Yield: 2.5 g (64 %)

                       $C_{17}H_{26}N_2O_4$ M.W. 322.4

   TLC Results: R_f:(S₁) 0

20   R_f:(S₄)   0.28

                       R_f:(S₅)   0.64

   HPLC Results: t_R 14.01 (min)

   FAB-MS: 323 (M+1)⁺

25   ## Example 8: Fmoc-Val Ψ[CH₂-N(CO-CH₂-CH₂-NHBoc)]Phe
      ## and Z-ValΨ[CH₂-N(CO-CH₂-CH₂-NHBoc)Phe-Obzl

   Z-ValΨ(CH₂-NH)Phe-Obzl (2.5 g, 4.9 mmol) were dissolved in 20 ml DMF and at 0 °C added consecutively with Boc-βAla (1.85 g, 9.8 mmol), HATU (3.72 g, 9.8 mmol), HOBt (1.33 g, 9.8 mmol) and DIEA (3.24 ml, 19.6 mmol). The reaction solution was

30 stirred 1 hour at 0 °C and overnight at room temperature. The solvent was removed under vacuum and the residue dissolved in 200 ml ethylacetate to provide a solution which was then washed with 0.1 M KHSO₄, with 0.1 M NaHCO₃ and with water. After drying over Na₂SO₄, the ethylacetate was removed.

   The resulting solid residue (3 g) contained both pseudodipeptide and Boc-βAla, and

35 so the crude product was recoupled with PyBrop. The reaction product was resolved in 120 ml CH₂Cl₂ and added to Boc-βAla (1.85 g), PyBrop (4.56 g, 9.8 mmol), and DIEA (4.86 ml, 29.6 mmol) at 0°C . The reaction mixture was stirred for 72 hours at room temperature. After the solvent was removed, the residue was dissolved in 200 ml ethylacetate and again

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extracted as described above. After the ethylacetate was removed, the crude reaction product

5   was dissolved in 60 ml methanol and precipitated with 0.6% aqueous acetic acid. The

precipitate shows a purity sufficient for further use in the following steps.

Yield: not determined

$C_{37}H_{47}N_3O_7$ M.W. 645.77

F. 183-185°C

10   $[\alpha]^{20} = -2.7 \pm 1$ (c= 1.0, CH_3OH)

$E_A(Arg) = 0$

TLC Results: $R_f:(S_2)$ 0.9

$R_f:(S_4)$ 0.75

$R_f:(S_5)$ 0.85

15   $R_f:(S_6)$ 0.8

HPLC Results: t_R: 53.5 min

FAB-MS: $[M^+]$: obsd. 646

Example 9: H-ValΨ[CH₂-N(CO-CH₂-CH₂-NHBoc)]Phe-OH

20   Z-ValΨ[CH$_2$-N(CO-CH$_2$-CH$_2$-NHBoc)]Phe-Obzl (0.5 g) was dissolved in 50 ml

acetic acid (95%) and hydrogenated in the presence of catalyst (palladium acetate). The

catalyst was added in three portions. After hydrogenation overnight the catalyst was

removed by filtration and the solution was evaporated to dryness. The triturated crude

product was extracted with ethyl ether, dissolved in n-butanol and washed with 0.1 M

25   KHSO$_4$. The butanol solution was washed with water and evaporated.

Yield: not determined

$C_{22}H_{35}N_3O_5$ M.W. 421.51

F. degradation

$[\alpha] = +12.9 \pm 1$ (c=1.0, CH_3OH)

30   $E_A(Arg) = 0$

TLC Results: $R_f:(S_2)$ 0.6

$R_f:(S_4)$ 0.2

$R_f:(S_5)$ 0.55

$R_f:(S_6)$ 0.3

35   HPLC Results: t_R: 31.4 min

FAB-MS: $[M^+]$: obsd. 422

Example 10: HN(CH$_2$-CH$_2$-COOAll)Phe-OBu^t

5   A solution of formyl Meldrum's acid (6.0g, 34.8mmol) and allylic alcohol (2.85ml, 41.8mmol) in dry toluene (60ml) was refluxed for 1.5 hours. The solvent was evaporated under vacuum and the crude product used without further purification.

      The crude product was dissolved in absolute methanol (30ml) and added to a solution of H-Phe-OBu^t·HAc (9.79g, 34.8mmol) and triethylamine (5.4ml, 34.8mmol) in dry

10 methanol (30ml) at 0 °C. The solution was stirred with 3 g molecular sieves at room temperature for 1hour, and then NaCNBH$_3$ (2.4g, 38.3mmol) was added at 0 °C. Stirring continued at room temperature for 12 hours. The reaction mixture was then filtered and the solvent was removed. The residue was taken up with ethyl acetate (200ml), washed with 5% KHSO$_4$ (6 x 600ml), 5% NaHCO$_3$ (3 x 100ml), saturated aqueous NaCl solution (3 x

15 100ml), dried over Na$_2$SO$_4$; and evaporated to dryness to yield a white powder.

Yield:	7.1g	
	C$_{19}$H$_{27}$NO$_4$	MW: 333.42
TLC Results:	R$_F$:	0.82 (hexane/ethyl acetate 1/1 v/v)
HPLC Results:	t$_R$:	27.7 min

20 FAB-MS: 334 (M+H)

Example 11: HN(CH$_2$-CH$_2$-CH$_2$-NH-Alloc)Phe-OBu^t

      18.0g (83.24mmol) Alloc-_Ala-N,O-dimethylhydroxamate was dissolved in 200ml dry ether and cooled to 0°C. To the clear solution 3.9g (103.9mmol) LiAlH$_3$ was added

25 carefully in small portions. The reaction mixture was stirred for 10 minutes at 0 °C and 40 minutes at room temperature. The reaction was finished with KHSO$_4$ (19.8g, 145.7mmol) in 100ml water at 0 °C. The ether phase was separated and the suspension of LiAlH$_3$/KHSO$_4$ was washed 3 times with ether (50ml). The ether phase was extracted with KHSO$_4$ (3 x 30ml), NaHCO$_3$ (3 x 50ml) and NaCl (3 x 50ml). The aldehyde (oil) was used as crude

30 product. Yield: 8.0g

      Phe-OBu^t·HAc (14.3g, 50.8mmol) was dissolved in dry methanol (50ml) cooled to 0 °C , triethylamine (7.0ml, 50.8mmol) and Alloc-ßAla-CHO (8.0g, 50.8mmol) in 50ml methanol and molecular sieve (2-3 g) were added. The reaction mixture was stirred for 1 hour at room temperature, cooled to 0 °C and then NaCNBH$_3$ (3.52g, 55.9mmol) was added

35 in small portions. The stirring was continued for 12 hours at room temperature. After removing the molecular sieves, the solvent was evaporated, the residue was taken up with ethyl acetate (150ml), washed with KHSO$_4$ 3 x 30ml), NaHCO$_3$ (3 x 50ml) and NaCl (3 x

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50ml). The organic phase was dried over Na_2SO_4 and the solvent removed by evaporation.

5 The crude product (17g) was washed with ether and hexane to get a white powder.

Yield:	7.8g	
	$C_{20}H_{30}N_2O_4$	MW: 362.46
TLC Results:	R_F:	0.44 (hexane/ethyl acetate 1/1 v/v)
		0.58 (chloroform/methanol 9/1 v/v)
		0.84 (n-butanol/HAc/water 4/1/1 v/v/v)
HPLC Results:	t_R:	27.14 min
FAB-MS:	363 (M+H)	

Example 12: Fmoc-DPhe_[CO-N(CH$_2$-CH$_2$-CH$_2$-NHAlloc)]Phe-OH

15 To a solution of NH(CH$_2$-CH$_2$-CH$_2$-NHAlloc)Phe-OBu^t (0.8g, 2.2mmol) in dry DCM were mixed 440µl collidine (3.3 mmol) and 1.8g Fmoc-DPhe-F (4.4mmol). The clear solution was stirred by 50 °C over 4 hours. The solvent was removed under vacuum and the crude product was resolved in ethyl acetate and extracted with 5% $KHSO_4$ (3x30ml), NaHCO$_3$ (3x30ml) and NaCl (3x30ml). The dry organic layer was evaporated

20 under vacuum.

Fmoc-DPhe_[CO-N(CH$_2$-CH$_2$-CH$_2$-NHAlloc)]Phe-OBu^t was purified by flash chromatography on silica gel 60 (MERCK) with pure chloroform. The fractions without Fmoc-DPhe-OH were collected and evaporated under vacuum. The resulting oil was treated with 50% TFA/DCM (and some drops of water) for 2 hours at room temperature. The

25 product (1.0g) was obtained as an oil and purified by flash chromatography on silica gel with chloroform /methanol 94/6.

Yield:	550mg (37%)	
	$C_{40}H_{41}N_3O_7$	MW: 675.75
TLC Results:	R_F:	0.42 (chloroform/methanol 9/1 v/v)
30 HPLC Results:	t_R	47.34 min
FAB-FAB-MS:	676 (M$^+$)	

35

Example 13: Fmoc-Val_[CO-N(CH$_2$-CH$_2$-COOAll)]Phe-OH

5   To a mixed solution of NH(CH$_2$-CH$_2$-COOAll)Phe-OBu^t (700 mg, 2.1 mmol) in DCM, 417 µl collidine (3.15mmol) and 1.5 g Fmoc-Val-F (4.12 mmol) were added. The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture was extracted with KHSO$_4$ (3 x 30 ml), NaHCO$_3$ (3 x 30ml) and NaCl (3 x 30ml). The dried organic phase was evaporated under vacuum, and the resulting oil was treated with

10   TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-solution was evaporated under vacuum and washed 3 times with ether. The crude product was obtained as an oil and purified by flash chromatography on silica gel 60 (MERCK) system 92/8 chloroform/methanol.

Yield:	1.96 g	
15	C$_{35}$H$_{38}$N$_2$O$_7$	MW: 598.67
	[ ]$_D^{25}$:	-75_1°(c=1, MeOH)
TLC Results: R$_F$:		0.46 (chloroform/methanol 9/1 v/v)
HPLC Results:	t$_R$	48.35 min
FAB-MS:	599.6 (M+H)	

20

Example 14: Fmoc-DVal_[CO-N(CH$_2$-CH$_2$-COOAll)Phe-OH

To a mixed solution of NH(CH$_2$-CH$_2$-COOAll)Phe-OBu^t (230 mg, 0.7 mmol) in DCM, 139 µl collidine (1.05 mmol) and 510 mg Fmoc-DVal-F (1.4mmol) were added. The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture

25   was extracted with KHSO$_4$ (3 x 30 ml), NaHCO$_3$ (3 x 30ml) and NaCl (3 x 30ml). The dried organic phase was evaporated under vacuum and the resulting oil was treated with TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-solution was evaporated under vacuum and washed 3 times with ether. The crude product was an oil which was purified by flash chromatography on silica gel 60 (MERCK) system

30   93/7 chloroform/methanol.

Yield:	523 mg	
	C$_{35}$H$_{38}$N$_2$O$_7$	MW: 598.67
	[ ]$_D^{25}$:	-34_1°(c=1, MeOH)
TLC Results:	R$_F$:	0.42 (chloroform/methanol 9/1 v/v)
35 HPLC Results:	t$_R$	47.76 min
FAB-MS:	599.9 (M+H) 597,5 (M-H)	

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Example 15: Fmoc-DVal [CO-N(CH₂-CH₂-CH₂-NHAlloc)Phe-OH

5   To a mixed solution of NH(CH₂-CH₂-CH₂-NHAlloc)Phe-OBu' (200 mg, 0.56 mmol)

in DCM, 100 µl collidine (0.84 mmol) and 400 mg Fmoc-DVal-F (1.12mmol) were added.

The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture

was extracted with KHSO₄ (3 x 30 ml), NaHCO₃ (3 x 30ml) and NaCl (3 x 30ml). The

dried organic phase was evaporated under vacuum and the resulting oil was treated with

10   TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-

solution was evaporated under vacuum and washed 3 times with ether. The crude product

was obtained as an oil and was purified by flash chromatography on silica gel 60 (MERCK)

system 94/6 chloroform/methanol.

Yield:	560 mg (mmol).	
15	$C_{36}H_{41}N_3O_7$	MW: 627.71
TLC Results:	R_F:	0.57 (chloroform/methanol 9/1 v/v)
HPLC Results:	t_R	45.47 min
FAB-MS:	5628.8 (M+H) 626.8 (M-H)	

20   Example 16: Fmoc-Val [CO-N(CH₂-CH₂-CH₂-NHAlloc)Phe-OH

To a mixed solution of NH(CH₂-CH₂-CH₂-NHAlloc)Phe-OBu' (2.1 g, 5.88 mmol) in

DCM, 1.19 ml collidine (8.82 mmol) and 4.2 g Fmoc-Val-F (11.8mmol) were added.

The clear solution was stirred at 50 °C bath temperature for 4 hours. The reaction mixture

was extracted with KHSO₄ (3 x 30 ml), NaHCO₃ (3 x 30ml) and NaCl (3 x 30ml). The dried

25   organic phase was evaporated under vacuum to yield an oil, which was then treated with

TFA/DCM 1:1 (and some drops of water) for 2 hours at room temperature. The TFA/DCM-

solution was evaporated under vacuum and washed 3 times with ether. The crude product

was obtained as an oil, which was purified by flash chromatography on silica gel 60

(MERCK), system chloroform/methanol 92/8.

30 Yield:	5.85 g	
	$C_{36}H_{41}N_3O_7$	MW: 627.71
TLC Results:	R_F:	0.70 (chloroform/methanol 9/1 v/v)
HPLC Results:	t_R	47.29 min
FAB-MS:	628.6 (M+H)	

35

Example 17: Fmoc-Val [CO-N(CH₂-CH₂-COOAll)]DPhe-OH

To a solution of NH(CH₂-CH₂-COOAll)DPhe -OBu' (1.0g, 3.0mmol) in dry DCM

were mixed 597µl collidine (4.5 mmol) and 2.15g Fmoc-Val-F (6.0mmol). The clear

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solution was stirred at 50 °C for 4 hours. The solvent was then removed under vacuum and the crude product was resolved in ethyl acetate and extracted with solution of 5% KHSO₄ (3x30ml), saturated NaHCO₃ (3x30ml), and saturated NaCl (3x30ml). The dried (Na₂SO₄) organic layer was evaporated under vacuum.

Fmoc-Val_[CO-N(CH₂-CH₂-COOAll)]DPhe-OBuᵗ was purified by flash chromatography on silica gel 60 (MERCK) with pure chloroform. The fractions without Fmoc-Val-OH were collected and evaporated under vacuum. The resulting oil was treated with 50 % TFA/DCM (and some drops of water) for 2hour at room temperature. The crude product (1.0g) was obtained as an oil, which was purified by flash chromatography on silica gel with chloroform /methanol 96/4.

Yield: 255 mg

$C_{35}H_{38}N_2O_7$ MW: 598.67

$[\ ]_D^{25}$ +30.68° _ 1 (c=1,MeOH)

TLC Results: R_F: 0.79 (CH₂Cl/methanol 9/1 v/v)

HPTLC-plates (Silica gel 60 F_{254}, Merck HPTLC-glass plates)

HPLC Results: t_R: HPLC   47.11 min

(Knauer, 220 nm, HD-SIL 18-5s-80, 250x4 mm, gradient from 20% acetonitrile containing 0.1% TFA to 80% acetonitrile, flow rate 1 ml/min)

FAB-FAB-MS: 599 (M⁻)

Example 18: Synthesis of Fmoc-Val-Hydroxamate

One equivalent of Fmoc-Val-OH (10.00 mmol; 3.4 g) and 1equivalent N,O-dimethylhydroxylamine hydrochloride (10.00 mmol; 1.08 g) were dissolved in 100 ml THF. The mixture as cooled to 0 °C, at which point 1.1 equivalent DIEA (11.00 mmol; 1.88 ml) and 1 equivalent N,N'-dicyclohexylcarbodiimid (10.00 mmol; 2.06 g) were added and stirring was continued at 0 °C for 1 hour, and then at room temperature for 4 hours. The precipitating dicyclohexyl urea was filtered off and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate and extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x). After drying with Na₂SO₄ and concentrating under vacuum, a wax-like substance was obtained. Remaining dicyclohexyl urea was separated using diethyl ether. The urea was filtered off, and the solvent removed under vacuum.

Yield:	93%		
	$C_{22}H_{26}N_2O_4$	M_w 382 g/mol	
TLC Results:	R_F	0.14	(hexane/ ethyl acetate 8:2)
	R_F	0.76	(benzene/acetone/acetic acid 27:10:0.5)
	R_F	1	(chloroform/ methanol 9:1)
HPLC Results:	t_R	38.10	(20-80% B in 60 min; Vydac C18)
FAB-MS:	$[MH]^+$ 383		

Example 19: Synthesis of Fmoc-Val-CHO

One equivalent Fmoc-Val-hydroxamate (13.07 mmol; 5.00 g) was dissolved in dry THF. This solution was evacuated and covered with argon. The mixture was then cooled to -15°C and 1.25 equivalents LiAlH$_4$ (16.33 mmol; 0.62 g) was carefully added in portions. Stirring was continued at -15°C for 15 min and then at room temperature for 1 hour. The mixture was then hydrolyzed with a concentrated solution of 1.75 equivalents KHSO$_4$ (22.87 mmol; 3.11 g), and the THF was evaporated under vacuum. The remaining aqueous solution was extracted with diethyl ether several times. The extracts were combined and extracted with KHSO$_4$ (3x), NaHCO$_3$ (3x) and NaCl (3x). After drying with Na$_2$SO$_4$ and concentrating under vacuum, an oil was obtained that was immediately used for further processing.

Yield:	60-80%		
	$C_{20}H_{21}NO_3$	M_w 323 g/mol	
TLC Results:	R_F	0.37	(hexane/ ethyl acetate 8:2)
	R_F	0.89	(benzene/acetone/ acetic acid 27:10:0.5)
	R_F	1	(chloroform/ methanol 9:1)
HPLC Results:	t_R	36.26	(20-80% B in 60 min; Vydac C18)
FAB-MS:	$[MH]^+$ 324		

Example 20: Synthesis of Fmoc-ValΨ[CH$_2$NH]Phe-OBu^t

One equivalent Phe-OBu^{t}xHAc (30.95 mmol; 8.72 g) was treated with 1 equivalent triethylamine (30.95 mmol; 4.31 ml) and was then added to a solution of 1 equivalent Fmoc-Val-CHO (30.95 mol; 10.00 g) in dry methanol with molecular sieves. This mixture was stirred for 1 hour before it was cooled to 0 °C, at which point 1.1 equivalent NaCNBH$_3$ (34.05 mmol; 2.14 g) was added in portions. Stirring was continued overnight. The

molecular sieves were filtered off and the methanol removed under vacuum to provide a

5   residue which was then dissolved in ethyl acetate and extracted with $KHSO_4$ (3x), $NaHCO_3$

(3x) and NaCl (3x). After drying with Na_2SO_4 and concentrating under vacuum, a white

solid compound was obtained.

Yield:	83% (crude product)		
	$C_{33}H_{40}N_2O_4$	M_w 528 g/mol	
TLC Results:	R_F	0.25	(hexane/ ethyl acetate 8:2)
	R_F	0.57	(chloroform/ methanol 9:1)
	R_F	0.88	(benzene/ aceton/ acetic acid 27:10:0.5)
HPLC Results:	t_R	42.21	(20-80% B in 60 min Vydac C18)
FAB-MS:	$[MH]^-$ 529		

15

Example 21: Synthesis of Fmoc-ValΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBuᵗ

One equivalent Alloc-β-Ala (2 mmol; 0.346 g) was dissolved in dry THF and cooled

to -15°C. One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1 equivalent isobutyl

chloroformate (2 mmol; 0.264 ml) were then added. After 10 minutes, a solution of 1

20   equivalent Fmoc-ValΨ[CH₂NH]Phe-OBuᵗ (2 mmol; 1.056 g) and 1 equivalent N-methyl

morpholine (2 mmol; 0.22 ml) in dry THF was added. The mixture was allowed to warm up

gently to room temperature. After stirring overnight, the THF was removed and the residue

dissolved in ethyl acetate, extracted with $KHSO_4$ (3x), $NaHCO_3$ (3x) and NaCl (3x). The

organic phase was dried with Na_2SO_4, and the solvent removed under vacuum to provide a

25   yellow oil.

Yield:	92% (crude product) about 35% content		
	$C_{40}H_{49}N_3O_7$	M_w 683 g/mol	
TLC Results:	R_F	0.15	(hexane/ ethyl acetate 8:2)
	R_F	0.86	(chloroform/ methanol 9:1)
	R_F	1.00	(benzene/acetone/acetic acid 27:10:0.5)
HPLC Results:	t_R	55.11	(20-80% B in 60 min Vydac C18)
FAB-MS:	$[MH]^-$ 684		

Example 22: Synthesis of Fmoc-ValΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OH

35   Fmoc-ValΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBuᵗ (2.82 g) was treated with 90%

TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether

several times and then purified by flash-chromatography with chloroform/methanol (9:1)

using silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

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Yield:	23.5% (662 mg)		
	$C_{36}H_{41}N_3O_7$	M_w 627 g/mol	
	$[\alpha_D]^{25}$	- 51.6° (c=1; methanol)	
TLC Results:	R_F	0	(hexane/ethyl acetate 8:2)
	R_F	0.18	(chloroform/methanol 9:1)
	R_F	0.28	(benzene/acetone/acetic acid 27:10:0.5)
HPLC Results:	t_R	42.46	(20-80% B in 60 min Vydac C18)
FAB-MS:	[MH]⁻ 628		

Example 23: Synthesis of Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OBuᵗ

Succinic anhydride (0.2 mol; 20.00 g), allyl alcohol (0.6 mol; 12.30 ml) and a catalytic amounts of DMAP were combined in 200 ml toluene and refluxed for 4 hours. The solvent and excessive allyl alcohol were removed by evaporation and the crude product was fractionated under vacuum.

Yield:	84%		
	$C_7H_{10}O_4$	M_w 158 g/mol	
K_p:	101°C at 0.072 mbar		
FAB-MS:	[MH]⁺ 159		

One equivalent succinic acid monoallylester (2 mmol; 0.316 g) was dissolved in dry THF and cooled to -15°C. One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1 equivalent isobutyl chloroformate (2 mmol; 0.264 ml) were then added. After 10 minutes, a solution of 1 equivalent Fmoc-ValΨ[CH₂NH]Phe-OBuᵗ (2 mmol; 1.056 g) and 1 equivalent N-methyl morpholine (2 mmol; 0.22 ml) in dry THF was added. The mixture was allowed to warm up gently to room temperature. After stirring overnight, the THF was removed and the residue dissolved in ethyl acetate, extracted with $KHSO_4$ (3x), $NaHCO_3$ (3x) and NaCl (3x). The organic phase was dried with Na_2SO_4 and the solvent was removed under vacuum to provide a yellow oil.

Yield:	95% (crude product) about 20% content		
	$C_{40}H_{39}N_3O_7$	M_w 659 g/mol	
TLC Results:	R_F	0.31	(hexane/ ethyl acetate 8:2)
	R_F	0.93	(chloroform/ methanol 9:1)
	R_F	1.00	(benzene/ aceton/ acetic acid 27:10:0.5)
HPLC Results:	t_R	58.54	(20-80% B in 60 min Vydac C18)
FAB-MS:	[MH]⁻ 660		

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Example 25: Synthesis of Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OH

Fmoc-ValΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OBuᵗ (2.40 g) was treated with 90% TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether several times and then purified by flash-chromatography with chloroform/methanol (9:1) using silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

Yield:	26% (583 mg)		
	$C_{36}H_{40}N_2O_7$	612 g/mol	
	$[\alpha_D]^{25}$	-49.9°	(c=1; methanol)
TLC Results:	R_F	0	(hexane/ethyl acetate 8:2)
	R_F	0.18	(chloroform/methanol 9:1)
	R_F	0.28	(benzene/acetone/acetic acid 27:10:05)
HPLC Results:	t_R	45.25	(20-80% B in 60 min Vydac C18)
FAB-MS:	[MH]⁺ 613		

Example 26: Synthesis of Fmoc-DPhe-Hydroxamate

One equivalent Fmoc-DPhe-OH (25.80 mmol; 10.00 g) and 1 equivalent N,O-dimethylhydroxylamine hydrochloride (28.38 mmol; 2.78 g) were dissolved in 100 ml THF, and the mixture was cooled to 0°C. To this were added 1.1 equivalents DIEA (28.38 mmol; 4.86 ml) and 1 equivalent N,N'-dicyclohexylcarbodiimid (25.80 mmol; 5.32 g), and stirring was continued at 0 °C for 1 hour and then at room temperature for 4 hours. The precipitating dicyclohexyl urea was filtered off and the solvent was removed under vacuum. The residue was dissolved in ethyl acetate and extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x). After drying with Na₂SO₄ and concentrating under vacuum, a wax-like substance was obtained. Remaining dicyclohexyl urea was separated using diethyl ether. The urea was filtered off and the solvent removed under vacuum.

Yield:	93%		
	$C_{26}H_{26}N_2O_4$	M_W 430 g/mol	
TLC Results:	R_F	0.09	(hexane/ ethyl acetate 8:2)
	R_F	0.86	(benzene/acetone/acetic acid 27:10:0.5)
	R_F	1	(chloroform/ methanol 9:1)
HPLC Results:	t_R	40.52	(20-80% B in 60 min Vydac C18)
FAB-MS:	[MH]⁺ 431		

Example 27: Synthesis of Fmoc-DPhe-CHO

One equivalent Fmoc-DPhe-hydroxamate (23.23 mmol; 10.00 g) was dissolved in dry THF, and the solution was evacuated, covered with argon, and cooled to -15°C. 1.25 equivalents $LiAlH_4$ (29.04 mmol; 1.11 g) were then carefully added in portions. Stirring was continued at -15°C for 15 min and then at room temperature for 1 hour. The mixture was then hydrolyzed with a concentrated solution of 1.75 equivalent $KHSO_4$ (40.65 mmol; 5.53 g). The THF was evaporated under vacuum. and the remaining aqueous solution was extracted with diethyl ether several times. The extracts were combined and extracted with $KHSO_4$ (3x), $NaHCO_3$ (3x) and NaCl (3x). After drying with Na_2SO_4 and concentrating under vacuum, an oil was obtained that was immediately used for further processing.

Yield:	60-80%		
	$C_{24}H_{21}NO_3$	M_w 355 g/mol	
TLC Results:	R_F	0.16	(hexane/ ethyl acetate 8:2)
	R_F	0.80	(benzene/acetone/acetic acid 27:10:0.5)
	R_F	1	(chloroform/ methanol 9:1)
HPLC Results:	t_R	35.57	(20-80% B in 60 min Vydac C18)
FAB-MS:	$[MH]^+$ 356		

Example 28: Synthesis of Fmoc-DPheΨ[CH₂NH]Phe-OBuᵗ

One equivalent Phe-OBuᵗxHAc (11.42 mmol; 3.22 g) was treated with 1 equivalent triethylamine (11.42 mmol; 1.60 ml) and then added to a solution of 1 equivalent Fmoc-DPhe-CHO (11.42 mmol; 4.06 g) in dry methanol with molecular sieves. This mixture was stirred for 1 hour before being cooled to 0 °C. 1.1 equivalents $NaCNBH_3$ (12.56 mmol; 0.70 g) were added in portions, and stirring was continued overnight. The molecular sieves were filtered off and methanol evaporated under vacuum. The residue was dissolved in ethyl acetate and extracted with $KHSO_4$ (3x), $NaHCO_3$ (3x) and NaCl (3x). After drying with Na_2SO_4 and concentrating under vacuum, a yellow oil was obtained.

Yield:	98% (crude product) 41% content		
	$C_{37}H_{40}N_2O_4$	M_w 576 g/mol	
TLC Results:	R_F	0.21	(hexane/ ethyl acetate 8:2)
	R_F	0.89	(benzene/acetone/acetic acid 27:10:0.5)
	R_F	0.73	(chloroform/ methanol 9:1)
HPLC Results:	t_R	43.98	(20-80% B in 60 min Vydac C18)
FAB-MS:	$[MH]^+$ 577		

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Example 29: Synthesis of Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBuᵗ

One equivalent Alloc-β-Ala (2 mmol; 0.346 g) is dissolved in dry THF and cooled to
-15°C. One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1 equivalent isobutyl
chloroformate (2 mmol; 0.264 ml) were then added. After 10 minutes, a solution of 1
equivalent Fmoc-DPheΨ[CH₂NH]Phe-OBuᵗ (2 mmol; 1.152 g) and 1 equivalent N-methyl
morpholine (2 mmol; 0.22 ml) in dry THF was added. The mixture was allowed to gently
warm up to room temperature. After stirring overnight, the THF was removed and the
residue dissolved in ethyl acetate, extracted with KHSO₄ (3x), NaHCO₃ (3x) and NaCl (3x).
The organic phase was dried with Na₂SO₄ and the solvent removed under vacuum to
provide a yellow oil.

Yield:	96% (crude product) about 31% content		
	$C_{44}H_{49}N_3O_7$	M_W 731 g/mol	
TLC Results:	R_F	0.09	(hexane/ ethyl acetate 8:2)
	R_F	0.85	(chloroform/ methanol 9:1)
	R_F	1.00	(benzene/acetone/acetic acid 27:10:0.5)
HPLC Results:	t_R	55.26	(20-80% B in 60 min Vydac C18)
FAB-MS:	$[MH]^+$ 732		

Example 30: Synthesis of Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OH

Fmoc-DPheΨ[CH₂N(COCH₂CH₂NHAlloc)]Phe-OBuᵗ (2.275 g) is treated with 90%
TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether
several times and purified by flash-chromatography with chloroform/methanol (9:1) using
silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

Yield:	27.5% (577 mg)		
	$C_{40}H_{41}N_3O_7$	M_W 675 g/mol	
	$[\alpha_D]^{25}$	- 32.6° (c=1; methanol)	
TLC Results:	R_F	0	(hexane/ethyl acetate 8:2)
	R_F	0.44	(chloroform/methanol 9:1)
	R_F	0.35	(benzene/acetone/acetic acid 27:10:0.5)
HPLC Results:	t_R	43.25	(20-80% B in 60 min Vydac C18)
FAB-MS:	$[MH]^-$ 676		

Example 31: Synthesis of Fmoc-DPheΨ[CH$_2$N(COCH$_2$CH$_2$COOAll)]Phe-OBu^t

5   Succinic anhydride (0.2 mol; 20.00 g), allyl alcohol (0.6 mol; 12.30 ml) and a catalytic amounts of DMAP in 200 ml toluene were refluxed for 4 hours. The solvent and excessive allyl alcohol were removed and the crude product fractionated under vacuum.

Yield: 84%

C$_7$H$_{10}$O$_4$ M$_W$ 158 g/mol

10 K$_p$:   101°C at 0.072 mbar

FAB-MS: [MH]$^+$ 159

One equivalent succinic acid monoallylester (2 mmol; 0.316 g) was dissolved in dry THF and cooled to -15°C. One equivalent N-methyl morpholine (2 mmol; 0.22 ml) and 1

15 equivalent isobutyl chloroformate (2 mmol; 0.264 ml) are added. After 10 min a solution of 1 equivalent Fmoc-DPheΨ[CH$_2$NH]Phe-OBu^t (2 mmol; 1.152 g) and 1 equivalent N-methyl morpholine (2 mmol; 0.22 ml) in dry THF were then added. The mixture was allowed to warm up gently to room temperature. After stirring overnight, the THF was removed and the residue was dissolved in ethyl acetate, extracted with KHSO$_4$ (3x), NaHCO$_3$ (3x) and

20 NaCl (3x). The organic phase is dried with Na$_2$SO$_4$ and the solvent was removed under vacuum to provide a yellow oil.

Yield: 92% (crude product) about 27% content

C$_{44}$H$_{48}$N$_2$O$_7$ M$_W$ 716 g/mol

TLC Results: R$_F$ 0.20   (hexane/ ethyl acetate 8:2)

25   R$_F$ 0.93   (chloroform/ methanol 9:1)

                       R$_F$ 1.00   (benzene/ aceton/ acetic acid 27:10:0.5)

HPLC Results: t$_R$ 58.67   (20-80% B in 60 min Vydac C18)

FAB-MS: [MH]$^-$ 717

30 ## Example 32: Synthesis of Fmoc-DPheΨ[CH$_2$N(COCH$_2$CH$_2$COOAll)]Phe-OH

Fmoc-DPheΨ[CH$_2$N(COCH$_2$CH$_2$COOAll)]Phe-OBu^t (2.19 g) was treated with 90% TFA/water for 1 hour. After removing the TFA, the residue was washed with diethyl ether several times and purified by flash-chromatography with chloroform/methanol (9:1) using silica gel 60 for column chromatography (particle size 0.040-0.063 mm).

35 Yield: 36% (727 mg)

C$_{40}$H$_{40}$N$_2$O$_7$ M$_W$ 660 g/mol

[α$_D$]^{25} - 23.5° (c=1; methanol)

TLC Results: R$_F$ 0   (hexane/ethyl acetate 8:2)

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	R_F	0.48	(chloroform/methanol 9:1)
	R_F	0.52	(benzene/acetone/acetic acid 27:10:0.5)
HPLC Results:	t_R	46.00	(20-80% B in 60 min Vydac C18)
FAB-MS:	$[MH]^+$ 661		

10   While the present invention has been described with respect to the particular
embodiments and examples provided above, it will be apparent to those skilled in the art
that various changes and modifications may be made without departing from the spirit and
scope of the invention as defined in the claims. Such modifications are intended to fall
within the scope of the appended claims.

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THE CLAIMS

1.   A building unit of Formula (I):

$$(I)\quad X\text{-}AA_m\text{-}U\text{-}AA_p\text{-}Y$$

wherein AA_m and AA_p are residues of natural or synthetic amino acids; U is $C^\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C^\alpha H_2 N)[CO(CH_2)_q W]$; C^α is a carbon atom and is the α carbon of AA_m if m > 0; W is NH-X or COY; X is a hydrogen atom of an amine or amide or an amine or amide protecting group; Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group; m is a number from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6.

2.   The building unit of claim 1 wherein AA_m and AA_n each comprises one or more residues of an amino acid selected from the group consisting of D-Phe, Phe, D-Val, and Val; and Y is selected from the group consisting of hydroxyl, alkoxy, substituted alkoxy, allyloxy, substituted allyloxy, aryloxy, substituted aryloxy, and trimethylsilyl ether.

3.   The building unit of claim 2 wherein X is selected from the group consisting of H, Boc, Z, Fmoc, and Alloc; and Y is selected from the group consisting of OMe, OEt, OBu^t, OBzl, and All.

4.   The building unit of claim 1, said building unit having the structure of Formula (II):

$$(II)\quad X\text{-}AA_m C^\alpha ON[(CH_2)_{n+1}\text{-}W]AA_p\text{-}Y$$

wherein n is a number from 0 to 4; m is a number from 0 to 4; and p is a number from 0 to 4.

5.   The building unit of claim 4, said building unit being selected from the group
consisting of:

HN(CH$_2$COOBu^t)Phe-OMe;

Fmoc-D-Phe-[CO-N(CH$_2$COOBu^t)]Phe-OH;

Fmoc-D-Phe-[CO-N(CH$_2$CH$_2$NHBoc)]Phe-OH;

Fmoc-Val-[CO-N(CH$_2$COOBu^t)]Phe-OH;

Fmoc-Val-[CO-N(CH$_2$-CH$_2$-CH$_2$-NHBoc)]Phe-OH;

Fmoc-Val-[CO-N(CH$_2$-CH$_2$-NHBoc)]Phe-OH;

Boc-βAla -(CH$_2$-NH)Phe-OH;

Fmoc-DPhe-[CO-N(CH$_2$-CH$_2$-CH$_2$-NHAlloc)]Phe-OH;

Fmoc-Val-[CO-N(CH$_2$-CH$_2$-COOAll)]Phe-OH;

Fmoc-DVal-[CO-N(CH$_2$-CH$_2$-COOAll)Phe-OH;

Fmoc-DVal-[CO-N(CH$_2$-CH$_2$-CH$_2$-NHAlloc)Phe-OH;

Fmoc-Val-[CO-N(CH$_2$-CH$_2$-CH$_2$-NHAlloc)Phe-OH; and

Fmoc-Val-[CO-N(CH$_2$-CH$_2$-COOAll)]DPhe-OH.

6.   The building unit of claim 1, said building unit having the structure of
Formula (III):

(III)   X-AA$_m$Ψ(C^{α}H$_2$N)[CO(CH$_2$)$_q$-W]AA$_p$-Y
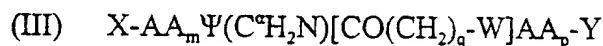
wherein n is a number from 0 to 3; m is a number from 0 to 4; and p is a number from 0 to
4, and q is a number from 0 to 4.

7.   The building unit of claim 6, said building unit being selected from the group
consisting of:

Fmoc-ValΨ[CH$_2$-N(CO-CH$_2$-CH$_2$-NHBoc)]Phe;

Z-ValΨ[CH$_2$-N(CO-CH$_2$-CH$_2$-NHBoc)Phe-Obzl;

H-ValΨ[CH$_2$-N(CO-CH$_2$-CH$_2$-NHBoc)]Phe-OH;

Fmoc-ValΨ[CH$_2$NH]Phe-OBu^t;

Fmoc-ValΨ[CH$_2$N(COCH$_2$CH$_2$NHAlloc)]Phe-OBu^t;

Fmoc-ValΨ[CH$_2$N(COCH$_2$CH$_2$NHAlloc)]Phe-OH;

Fmoc-ValΨ[CH$_2$N(COCH$_2$CH$_2$COOAll)]Phe-OBu^t;

Fmoc-ValΨ[CH$_2$N(COCH$_2$CH$_2$COOAll)]Phe-OH;

Fmoc-DPheΨ[CH$_2$N(COCH$_2$CH$_2$NHAlloc)]Phe-OBu^t;

Fmoc-DPheΨ[CH$_2$N(COCH$_2$CH$_2$NHAlloc)]Phe-OH;

Fmoc-DPheΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OBuᵗ; and

5   Fmoc-DPheΨ[CH₂N(COCH₂CH₂COOAll)]Phe-OH.

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5   8.   A method of synthesizing a building unit of Formula (II):

         (II) $X\text{-}AA_mC^\alpha ON[(CH_2)_{n+1}\text{-}W]AA_p\text{-}Y$

10 wherein AA_m and AA_p are residues of natural or synthetic amino acids; C^α is the α carbon of AA_m if m > 0 or else is simply a carbon atom; W is NH-X or COY; X is a hydrogen atom of an amine or amide or an amine or amide protecting group; Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group; m is a number from 0 to 10; n is a number from 0 to 6; and p is a number from 1 to 10; said method comprising:

         preparing a first reaction mixture comprising a compound of Formula (d):

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$$W-(CH_2)_n-\overset{\displaystyle \overset{H}{\underset{|}{N}}}{} AA_p-E$$
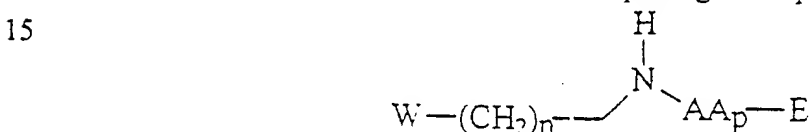
Formula (d)

wherein E is a carboxyl protecting group and W, N and AA_p are as defined above; and

20   reacting said first reaction mixture with a compound of Formula (e):

$$X-AA_m-\overset{\displaystyle O}{\overset{\|}{C}}-Hal$$
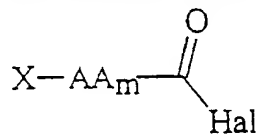
Formula (e)

wherein Hal is a halogen and X and m are as defined above, under reaction conditions
25 sufficient to form the building unit of Formula (II).

         9.   The method of claim 8 which further comprises selecting Hal to be F or Cl; selecting X from the group consisting of H, Boc, Z, Fmoc, and Alloc; and selecting Y from the group consisting of OMe, OEt, OBu', OBzl, and All.
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         10.   The method of claim 8 or 9 which further comprises utilizing a catalyst to facilitate the reaction of the first reaction mixture with the compound of Formula (e), said catalyst being selected from the group consisting of collidine, BSTA, HBTU, HOBT, DIEA, and mixtures thereof.
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         11.   The method of claim 8 to 10 which further comprises removal of the protecting group E after formation of the building unit of Formula (II).

12.   The method of any one of claims 8 to 11 wherein the first reaction mixture is prepared by reacting a compound of Formula (b):
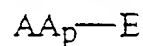
$$W-(CH_2)_n-\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}-H$$
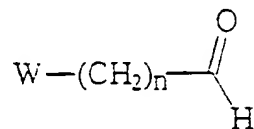
Formula (b)

where W and n are as defined above, with a compound of Formula (c):
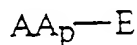
$$AA_p-E$$

Formula (c)

where AA_p and E are as defined above, under reaction conditions sufficient to form the compound of Formula (d).

13.   The method of claim 12 which further comprises obtaining the compound of Formula (b) from cleavage of an N-alkoxyamide.

14.   The method of claim 13 which further comprises selecting the N-alkoxyamide to be a compound of Formula (a):

$$W-(CH_2)_n-\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}-\underset{\displaystyle |}{N}-OCH_3$$
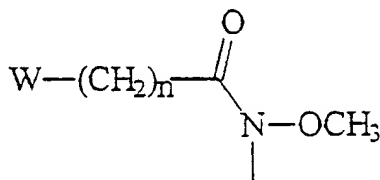
Formula (a)

15.   The method of any one of claims 8 to 11 wherein the first reaction mixture is prepared by reacting a compound of Formula (x):

$$O=\overset{}{\diagup}\diagdown\overset{\displaystyle O}{\overset{\displaystyle \|}{C}}-OE'$$
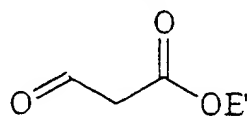
Formula (x)

wherein E' is a carbonyl protecting group, with a compound of Formula (c):
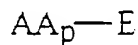
$$AA_p-E$$

Formula (c)

where AA_p and E are as defined above, under reaction conditions sufficient to form the compound of Formula (d).

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16.   The method of claim 15 wherein the compound of Formula (j) is prepared by rearrangement of a compound of Formula (w):
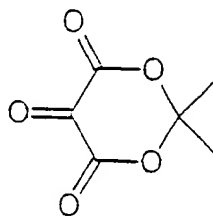
Formula (w)

17.   A method of synthesizing a building unit of Formula (III):
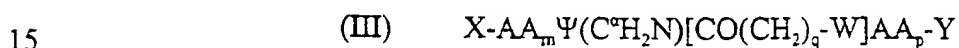
(III) $X\text{-}AA_m\Psi(C^{\epsilon}H_2N)[CO(CH_2)_q\text{-}W]AA_p\text{-}Y$

wherein AA_m and AA_p are residues of natural or synthetic amino acids; C^{ϵ} is the α carbon of AA_m if m > 0 or else is simply a carbon atom; W is NH-X or COOY; X is a hydrogen atom of an amine or amide or an amine or amide protecting group; Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group; m is a number from 0 to 10; p is a number from 1 to 10; and q is a number from 1 to 6; said method comprising:

preparing a first reaction mixture comprising a compound of Formula (m):
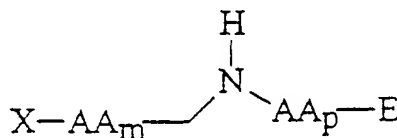
Formula (m)

wherein E is a carboxyl protecting group and X, AA_m and AA_p are as defined above; and reacting said first reaction mixture with a compound of Formula (n):
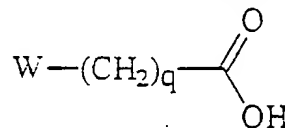
Formula (n)

where W and q are as defined above, under reaction conditions sufficient to form the building unit of Formula (III).

18.   The method of claim 17 which further comprises selecting X from the group consisting of H, Boc, Z, Fmoc, and Alloc; and selecting Y from the group consisting of OMe, OEt, OBu^t, OBzl, and All.

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19.   The method of claim 17 or 18 which further comprises utilizing a catalyst to facilitate the reaction of the first reaction mixture and the compound of Formula (n), said catalyst being selected from the group consisting of collidine, BSTA, HBTU, HOBT, DIEA, and mixtures thereof.

20.   The method of any one of claims 17 to 19 which further comprises removal of the protecting group E after formation of the building unit of Formula (III).

21.   The method of any one of claims 17 to 20 wherein the first reaction mixture is prepared by reacting a compound of Formula (k):

$$X-AA_m-\overset{O}{\underset{H}{\diagup\diagdown}}$$
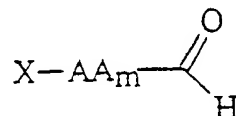
Formula (k)

where X and m are as defined above, with a compound of Formula (c):

$$AA_p-E$$

Formula (c)

where AA_p and E are as defined above, under reaction conditions sufficient to form the compound of Formula (m).

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SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(54) Title: BRANCHED BUILDING UNITS FOR SYNTHESIZING CYCLIC PEPTIDES

(57) Abstract

The present invention relates to building units suitable for the synthesis of backbone cyclized peptides. The building units of this invention have structures of the formula (I): $X–AA_m–U–AA_p–Y$ wherein AA_m and AA_p are residues of natural or synthetic amino acids and U is $C_\alpha ON[(CH_2)_{n+1}W]$ or $\Psi(C_\alpha H_2 N)[CO(CH_2)_q W]$, in which C_α is a carbon atom and is the α carbon of AA_m if $m > 0$. W is NH–X or COY, and X is H, Boc, Z, Fmoc, or Alloc while Y is the hydroxyl group of a carboxylic acid moiety or a carboxylic acid protecting group. The letter m refers to an integer from 0 to 10; n is a number from 0 to 6; p is a number from 1 to 10; and q is a number from 1 to 6. The present invention is also directed to methods of synthesizing building units of formula (I) via different routes.

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6   C07K5/02   C07K5/06   C07C271/22   C07C271/20   C07C229/36
        C07C237/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   C07K   C07C

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. BYK ET AL: "Building Units for N-backbone cyclic peptides. 1." J. ORG. CHEM., vol. 57, 1992, pages 5687-5692, XP002100997 cited in the application	1-4,6
Y	*Schemes I, IV, especially scheme V* see page 5690	8-21
X	G. BITAN ET AL.: "Building Units for N-backbone cyclic peptides. 2." TETRAHEDRON, vol. 51, no. 38, 1995, pages 10513-10522, XP002100998 *Scheme 5*	1-4,6
	—/—	

| X | Further documents are listed in the continuation of box C. | | X | Patent family members are listed in annex. |

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 1999	06/07/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Cervigni, S

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category°	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
X	D. MÜLLER ET AL.: "Building Units for N-backbone cyclic peptides. 3." J. ORG. CHEM., vol. 62, no. 2, January 1997, pages 411-416, XP002100999 *compound 11*	1-6
X	G. BITAN ET AL.: "Building Units for N-backbone cyclic peptides. 4." J. CHEM. SOC. PERKIN TRANSACTION 1, no. 10, May 1997, pages 1501-1510, XP002101000	1-4,6
Y	*Schemes 1-3*	8-21
X	EP 0 564 739 A (YISSUM RES DEV CO (IL))) 13 October 1993 see claims	1-4,6
X	WO 95 33765 A (PEPTOR LTD ;YISSUM RES DEV CO (IL); GILON CHAIM (IL); EREN DORON ()) 14 December 1995	1-4,6
Y	see page 41; claims	8-21
A	WO 97 09344 A (PEPTOR LIMITED ;YISSUM RES DEV CO (IL); HORNIK VERED (IL); GILON C) 13 March 1997	
A	KALJUSTE K ET AL: "A NEW GENERAL SOLID-PHASE METHOD FOR THE SYNTHESIS OF BACKBONE-TO- BACKBONE CYCLIZED PEPTIDES" INTERNATIONAL JOURNAL OF PEPTIDE AND PROTEIN RESEARCH, vol. 43, no. 5, 1 May 1994, pages 505-511, XP000440871 cited in the application	
Y	J. JONES: "Amino acids and peptide synthesis" 1992 , OXFORD SCIENCE XP002101001 see page 25 - page 41	8-21

1

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INTERNATIONAL SEARCH REPORT

Information on patent family members

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			JP	11500741 T	19-01-1999
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